

Human PCSK9/Proprotein Convertase 9 Sandwich ELISA Kit Datasheet

For the quantitative detection of human PCSK9/Proprotein Convertase 9 concentrations in serum, plasma, cell culture supernatants and cell lysates.

General Information

Catalogue Number	KE00181
Product Name	Human PCSK9/Proprotein Convertase 9 Sandwich ELISA Kit
Species cross-reactivity	Human
Range (calibration Range)	0.156-10 ng/mL
Tested applications	Quantification ELISA

Database Links

Entrez Gene	255738
SwissProt	Q8NBP7

Kit Components & Storage

Microplate - antibody coated 96-well microplate (8 well × 12 strips)	1 plate	Unopened Kit: Store at 2-8°C for 6 months or -20°C for 12 months. Opened Kit: All reagents stored at 2-8°C for 7 days. Please use a new standard for each assay.
Protein standard - 10 ng/bottle; lyophilized*	2 bottles	
Detection antibody, biotinylated (100X) - 120 µL/vial	1 vial	
Streptavidin-horseradish peroxidase (HRP) (100X) - 120 µL/vial	1 vial	
Sample Diluent PT 1-ec - 30 mL/bottle. For serum, plasma and cell culture supernatants samples	1 bottle	
Sample Diluent PT 3-ec - 30 mL/bottle. For cell lysates	1 bottle	
Detection Diluent - 30 mL/bottle	1 bottle	
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	
Extraction Reagent - 30 mL/bottle	1 bottle	
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	
Stop Solution - 12 mL/bottle	1 bottle	
Plate Cover Seals	3 pieces	

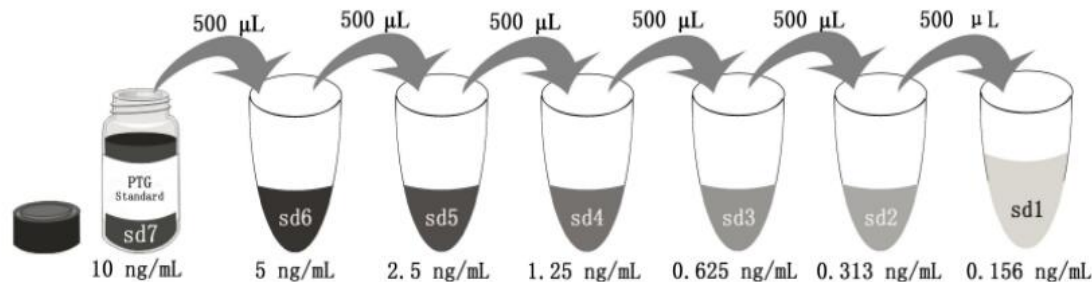
NB: Do not use the kit after the expiration date.

Sample Diluent PT 1-ec is for protein standard, serum, plasma and cell culture supernatants samples.

Sample Diluent PT 3-ec is for protein standard and cell lysates.

Detection Diluent is for Detection antibody and Streptavidin-HRP.

*Add 1 mL Sample Diluent PT 1-ec or PT 3-ec in protein standard. This reconstitution gives a stock solution of 10 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 1-ec or PT 3-ec	1000 µL	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product Description

KE00181 is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (Sandwich ELISA). The PCSK9 ELISA kit is to be used to detect and quantify protein levels of endogenous PCSK9. The assay recognizes human PCSK9. An antibody specific for PCSK9 has been pre-coated onto the microwells. The PCSK9 protein in samples is captured by the coated antibody after incubation.

Following extensive washing, another antibody of biotinylated specific for PCSK9 is added to detect the captured PCSK9 protein.

For signal development, Streptavidin-HRP is added, followed by Tetramethyl-benzidine (TMB) reagent. Solution containing sulfuric acid is used to stop color development and the color intensity which is proportional to the quantity of bound protein is measurable at 450 nm with the correction wavelength set at 630 nm.

Background

PCSK9, also named as NARC1 and PC9, belongs to the peptidase S8 family. PCSK9 is expressed and secreted by several tissues but principally by the liver, small intestines, and kidney(PMID 28587771). It may be implicated in the differentiation of cortical neurons and may play a role in cholesterol homeostasis. PCSK9 is a regulator of the LDL receptor and plasma cholesterol concentrations(PMID: 26293463).

Sample Preparation

The serum or plasma samples may require proper dilution to fall within the range of the assay. 1:40 or 1:80 dilution is recommended for serum or plasma. 1:20 or 1:40 dilution is recommended for cell culture supernatants. 1:10 or 1:20 dilution is recommended for cell lysates.

Safety Notes

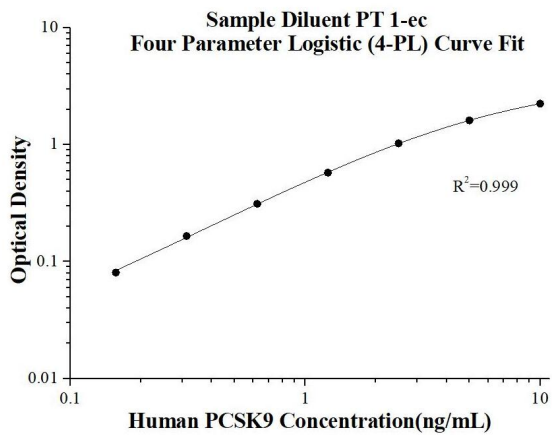
This product is sold for lab research and development use ONLY and not for use in humans or animals. Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay Procedure Summary

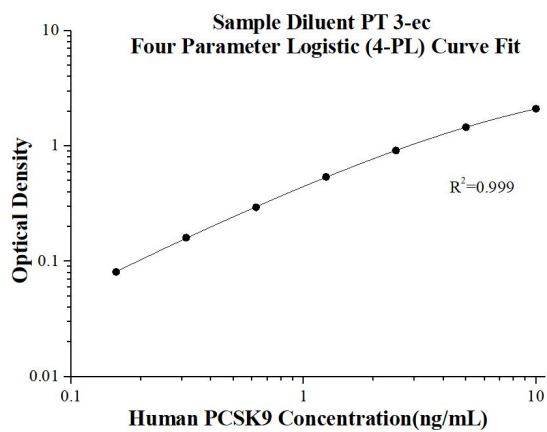
Step	Reagent	Volume	Incubation	Wash	Notes
1	Standard and Samples	100 μ L	120 min	4 times	Cover Wells incubate at 37°C
2	Diluent Antibody Solution	100 μ L	60 min	4 times	Cover Wells incubate at 37°C
3	Diluent HRP Solution	100 μ L	40 min	4 times	Cover Wells incubate at 37°C
4	TMB Substrate	100 μ L	15-20 min	Do not wash	Incubate in the dark at 37°C
5	Stop Solution	100 μ L	0 min	Do not wash	-
6	Read plate at 450 nm and 630 nm immediately after adding Stop solution. DO NOT exceed 5 minutes.				

Example data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average	Corrected
0	0.040 0.038	0.039	-
0.156	0.122 0.117	0.120	0.080
0.313	0.208 0.201	0.204	0.165
0.625	0.350 0.351	0.350	0.311
1.25	0.611 0.620	0.615	0.576
2.5	1.077 1.054	1.065	1.026
5	1.659 1.646	1.652	1.613
10	2.313 2.252	2.282	2.243



(ng/mL)	O.D	Average	Corrected
0	0.046 0.045	0.045	-
0.156	0.127 0.126	0.126	0.081
0.313	0.208 0.204	0.206	0.160
0.625	0.341 0.339	0.340	0.294
1.25	0.581 0.590	0.585	0.540
2.5	0.956 0.966	0.961	0.915
5	1.501 1.507	1.504	1.458
10	2.123 2.180	2.151	2.105

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

Intra-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	20	4.66	0.15	3.1
2	20	1.26	0.02	1.9
3	20	0.31	0.01	3.4

Inter-assay Precision				
Sample	n	Mean (ng/mL)	SD	CV%
1	24	4.91	0.23	4.6
2	24	1.26	0.05	3.8
3	24	0.27	0.02	6.7

Recovery

The recovery of PCSK9 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:80	96	91-105
	1:160	89	82-92
Cell culture supernatants	1:40	86	79-95
	1:80	88	79-104
Cell lysates	1:20	105	86-115
	1:40	104	87-111

Sample Values

Human serum, plasma samples from healthy volunteers were evaluated for PCSK9 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human serum (n=16)	67.2	13.1-155.5
Human plasma (n=16)	101.7	9.2-287.2

cell culture supernatants:

HepG2 (human hepatocellular carcinoma cells) were cultured in DMEM supplemented with 10% fetal bovine serum, 2.5 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. An aliquot of the cell culture supernatants was removed, assayed for human PCSK9, and measured 60.1 ng/mL.

cell lysates:

	HepG2 lysate
PCSK9 /Total protein (ng/mg)	3.6

Sensitivity

The minimum detectable dose of human PCSK9 is 0.01 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay. (The plasma and serum samples were initially diluted 1:20, the cell culture supernatants sample was initially diluted 1:10, the cell lysates sample was initially diluted 1:5)

		Human serum (PT 1-ec)	Cell culture supernatants (PT 1-ec)	Cell lysates (PT 3-ec)
1:2	Average% of Expected	100	100	100
	Range (%)	-	-	-
1:4	Average% of Expected	94	95	93
	Range (%)	89-98	94-97	88-97
1:8	Average% of Expected	88	92	94
	Range (%)	84-94	88-95	86-107
1:16	Average% of Expected	94	91	81
	Range (%)	91-97	88-97	78-86

References

1. Zhu YM. et al. (2015) *Arterioscler Thromb Vasc Biol.* 35(10):2254-2259.
2. Melendez QM. et al. (2017) *Arch Biochem Biophys.* 625-626:39-53.
3. Li JJ, Li S. et al. (2015) *Medicine (Baltimore).* 94(52):e2426.